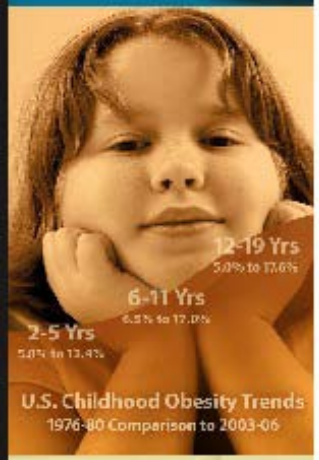
NTP
National Toxicology Program



NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity

Breakout Group on

Organotins and Phthalates

Kim Boekelheide (chair)
Kembra Howdeshell (rapporteur)

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DEPARTMENT OF HEALTH & HUMAN SERVICES • USA

Organotins + Phthalates Breakout Group Members

Bruce Blumberg, UC-Irvine
Kim Boekelheide, Brown U (chair)
Elizabeth Hatch, Boston U
Kembra Howdeshell, NIH/NIEHS/NTP (rapporteur)
Juliette Legler, Vrije University Amsterdam
Jennifer Schlezinger, Boston U

Background

Phthalates and **organotins** are grouped together because they both interact with the protein transcription factor PPARγ which is intimately involved in the regulation of adipocyte differention, metabolic syndrome, and insulin sensitivity. Both have a common use as plasticizers in polyvinylchloride (PVC) plastics and there are co-exposures to these two chemical classes.

Human Studies - Phthalates

- Current data from human studies of exposure to **phthalates** provide insufficient evidence of an association with diabetes or obesity.
- 3 papers: cross-sectional studies, NHANES data (2), Denmark (1), some positive associations
- These are exploratory epidemiology studies with preliminary data that suggest the possibility of gender differences in response, and that different phthalates may have different activities
- Active versus inactive phthalate congeners of phthalates: for anti-androgenic effects, mono-ethyl phthalate (MEP) is inactive, and therefore has not been studied very much. Does MEP bind to PPARγ? What about other phthalate metabolites?

Human Studies – Phthalates cont.

- Body weights tend to be decreased at the highest doses administered in toxicological studies (interesting mechanistic explanation for this!)
- Lack of information regarding other metabolic endpoints
- Route of exposure primarily oral via food, although some are dermal (i.e. MEP in cosmetics)
- Need to separate out the effect of obese individuals consuming more food and thus taking in more phthalates
- Adjust for BMI, dietary intake, % total intake of fat in diet

Human Studies - Organotins

- Current data from human studies of exposure to **organotins** are non-existent regarding an association with diabetes or obesity.
- There are no epidemiological data for the organotins, just a couple case reports.
- One study of accidental exposure to an organotins suggested an association between acute exposure and hyperglycemia and suggested a half-life of days. This in contrast to the half-life for phthalates, which is in hours.

Human Studies - Organotins

- Organotins are pesticides, in PVC products, anti-fouling agents on big ships
- Prioritize the organotins to study by current use
- Triphenyltin (Fentin), Phenbutatin, butyltins (used in plastics, including: mostly mono- and di, but look at all).
  - Highest priority: triphenyltin as it is used in agriculture. Tributyltin is still present in harbors and thus fish consumption results in exposure; continues to be used on very large ships. Is not volatile, but could be in house dust due to paint powdering.

Human Studies cont.

- There are not enough data for **phthalates** or **organotins** to determine a consistent association between chemical exposure and diabetes and/or obesity.

Most Useful Health Measures

- Most useful indicators of exposure and health diagnosis for **phthalates** and **organotins**:
  - Adiposity is the most studied endpoint
  - Multiple fat depots and significance of the different locations in terms of health effects
  - Waist circumference
  - Skin thickness
  - BMI above a certain standard
  - Hormone biomarkers (i.e. leptin) – would be useful, not currently employed
  - Triglycerides and cholesterol – lipids associated with PPARα and γ activation
  - Glucose tolerance, fasting glucose as a marker for diabetes

Most Useful Health Measures cont.

- Bone density – serum measures of calcitonin, osteoclastogenesis
- PPARγ is involved in different ways in different cell populations having complex effects on obesity
- DXA – preferred measure of bone density for osteopenia and osteoporosis
- bone-specific alkaline phosphatase.

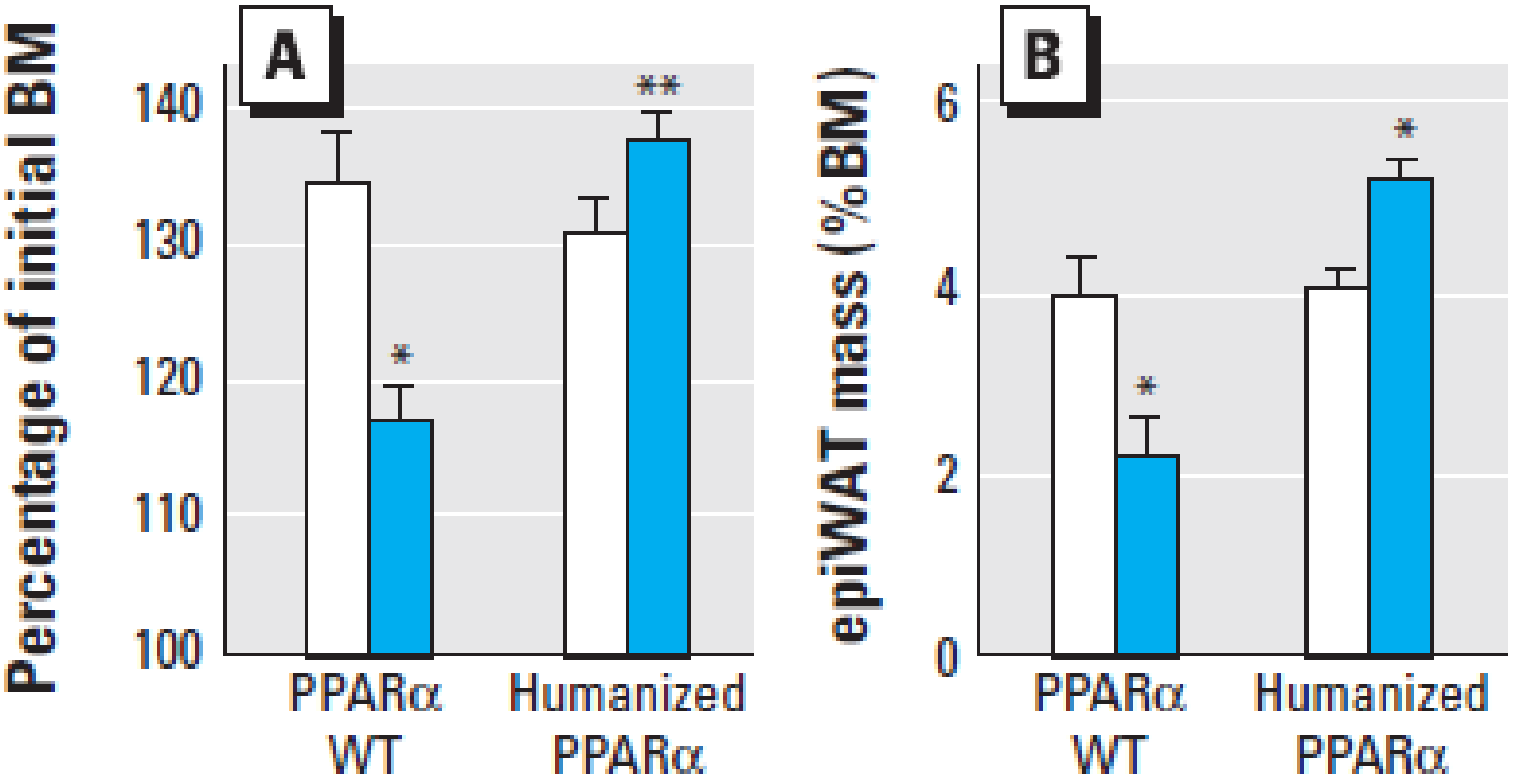
Adjustment Variables for Epidemiology Studies

- Those factors impacting development origins of diabetes and obesity:
  - Maternal BMI
  - Maternal weight gain – can affect birth weight
  - Maternal diabetes – gestational or type II
  - Maternal diet
- Infant diet – breast feeding versus formula feeding, introduction of food during nursing period
- Childhood diet
- Childhood physical activity
- Socio-economic variables
- Maternal smoking while pregnant
- Maternal age

Animal/Mechanistic Data - Phthalates

- Essential to pay attention to differences in PPARα activity between humans vs rodents with regard to body weight gain and other endpoints
  - difference between wildtype mouse and PPARα humanized mouse
- PPARγ in mouse and human act similarly
- PPARα in rat/mouse is strong and may mask PPARγ effect

Phthalates – Rodent vs Human PPARα

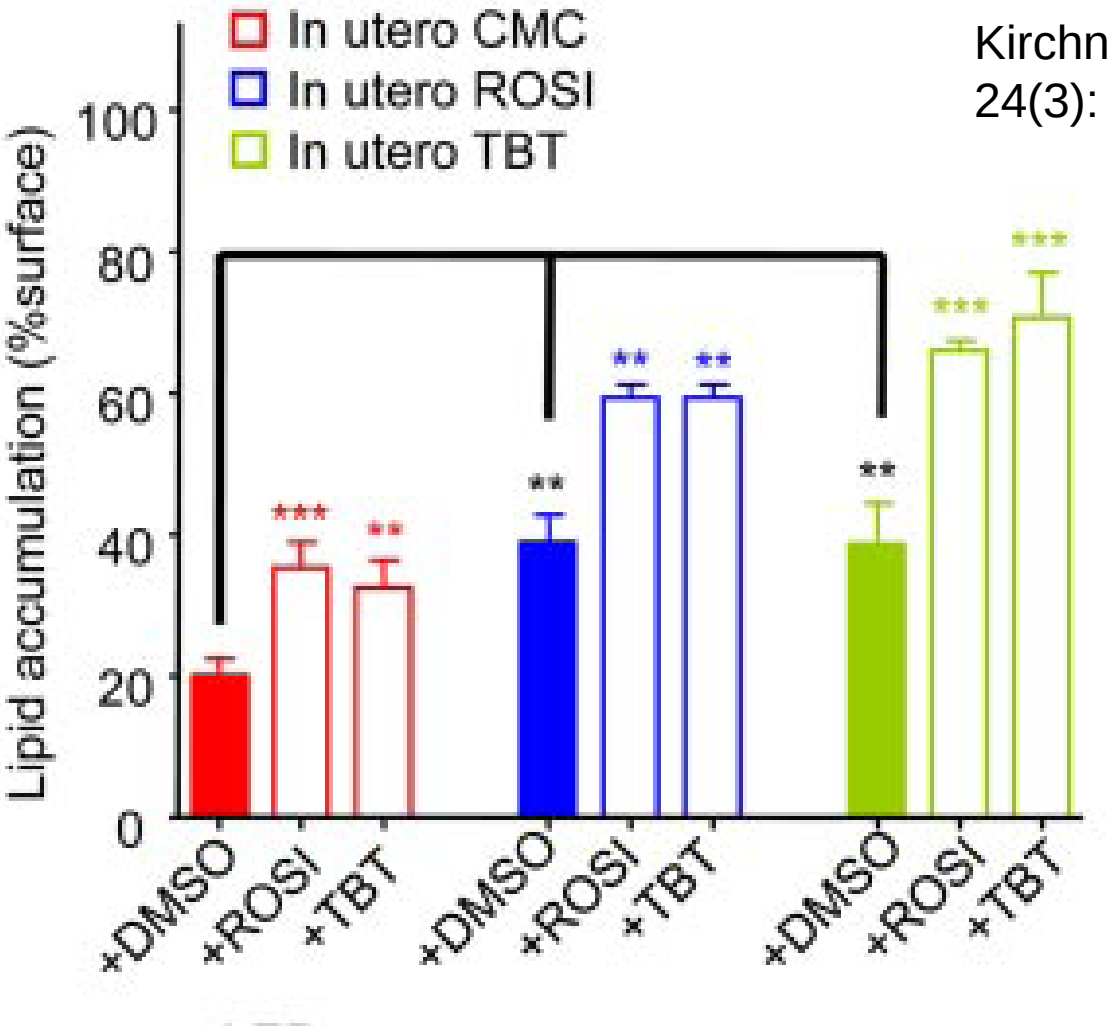


Feige J (2010) Environmental Health Perspectives 118(2): 234-241

Animal/Mechanistic Data - Organotins

- Few studies, but quality is good
- Relatively new in terms of diabetes and obesity studies
- Older immunotox literature not useful for obesity and diabetes, since studies were acute and high dose
- Environmental toxicology literature – exposure (in ppb) of aquatic organisms is similar to human exposure

Effect of TBT Prenatal Exposure on Adipogenic Capacities of Mouse Adipose Derived Stem Cells



Kirchner S (2010) Mol Endocrinol 24(3): 526-539

Animal/Mechanistic Data (cont)

- *In vitro* data can be very strong because these compounds are known ligands for known receptors
- ToxCast™ data is confusing, with inconsistent responses among the 3 tests
  - ATG test appears problematic
  - Tests did not pick up known positive PPAR agonists
  - Too high concentrations used?
  - Toxicity issues?
  - Potency does hold up across the 3 assays
  - Independent data need to be replicated

Summary

- Phthalates activate PPARγ at ~10-100 μM
- Organotins activate PPARγ at ~10-100 nM
- Exposure to phthalates is relatively high and ubiquitous
- Exposure to organotins is probably lower, but currently unknown
- Possibility of complex mixture effects because of the interactions between receptor systems

Data Needs

- Explore a broader range of phthalates in terms of receptor activation. Move beyond the anti-androgenic effects of phthalates and beyond only those phthalates that are anti-androgenic. For example, MEP has not been adequately assessed.
  - Do anti-androgenic activities of phthalates play into induction of metabolic disease?
- Human exposure data on the organotins
  - Measure leaching of organotins from products as a source of human exposure

Data Needs

- Consequences of developmental exposure in humans due to *in utero* and childhood exposure to chemicals (most studies evaluate adults)
- Biomarkers across life stages
  - Epigenetic biomarkers of *in utero* exposure
  - DNA methylation arrays for animal models and people.
- Timing of human exposure assessments
  - Need to know more about biology of adipocytes and their sensitive periods
  - Look from preconception through end of puberty

Data Needs

- Measure more, model better! – Improve computer modeling to determine how much people are exposed to (i.e., intake amounts) by incorporating more actual human/animal data. Challenges to measuring more: cost and compliance.
- Sensitive analytical methods to measure organotins, accessibility to measurements, non-invasive measurements (i.e., urine or saliva).
- Look at occupational exposures for organotins

Data Needs

- Low dose, developmental studies in animals on induction of obesity/diabetes with molecular mechanistic biomarkers of persistent effect
  - Always keep interaction with diet in mind
- Information on phthalate plus organotin mixture effects
- Improved understanding of the molecular biology of receptor activation
  - Interactions between receptor systems (i.e., organotins may activate RXR and phthalates activate PPARγ, and these receptors interact)
  - For PPARγ, phosphorylation events versus activity as a transcription factor

Data Needs

- PPARγ programs fat cells – how is the fat cell programmed? How is obesity programmed?
- Investigate whether there is an association between bone marrow adipogenesis, bone strength and immune function for both organotins and phthalates
  - Does prenatal exposure lead to increased fat in bone and increased bone fragility?
- Look at more than visceral fat, locations of other fat depots

Data Needs

- Do isoforms and SNPs of PPARγ in humans create populations particularly susceptible to xenobiotic exposures and obesity/metabolic syndrome?
- Evaluate comparative (aquatic organism) effects and studies for consistency with mammalian literature on organotins

Data Needs

- Better *in vitro* tests
  - More attention to the development of *in vitro* assays for molecular targets of concern
  - Develop integrative in vitro assays that measure development of fat depots in cell culture, a true biological endpoint of complex intersecting pathways
    - 3T3L1 cells are a good model for this effect and can be studied in a 96-well format